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A new reagent, 2-[phenyl(methyl)sulfonio]ethyl-4-nitro-phenylcarbonate tetrafluoroborate (Pms-ONp), for preparing water-soluble *N*-protected amino acids

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Abstract—Peptide syntheses are performed in various organic solvents, the disposal of which is an environmental problem. To avoid this problem, peptide synthesis in water using reagents of low toxicity is desirable. For peptide synthesis in water, we previously reported the design of a water-soluble *N*-protecting group, 2-[phenyl(methyl)sulfonio]ethoxycarbonyl tetrafluoroborate (Pms) group, but the introduction of this group onto sulfur-containing amino acids was problematic. Here, a new reagent, 2-[phenyl(methyl)sulfonio]ethyl-4-nitrophenylcarbonate tetrafluoroborate (Pms-ONp), has been designed and used to prepare Pms derivatives of sulfur-containing amino acids. Pms-Met was prepared and tested for the solid-phase synthesis of Met-enkephalin amide in water using a crosslinked ethoxylate acrylate resin.

Solid-phase peptide synthesis was originally devised by Merrifield,[1] and its many advantages have led not only to automatic peptide synthesis but also to the development of combinatorial chemistry. The synthetic procedure of solid-phase synthesis is simple but it requires a large amount of organic solvent. Because the safe disposal of organic solvents is an important environmental issue, our aim is to perform peptide synthesis in water. For this purpose, protected amino acids that are soluble in water must be generated. Previously,[2] we reported 2-[phenyl(methyl)sulfonio]ethoxycarbonyl (Pms) group as a water-soluble *N*-protecting group. Pms-amino acid derivatives are readily soluble in water and the Pms group is easily removable by treatment with mild bases. Pms amino acids were prepared according to route A, as shown in Scheme 1. 2-(Phenylthio)ethyl chloroformate **2** was prepared from 2-(phenylthio)ethanol **1** and phosgene (prepared from triphosgen)[3] in dichloromethane. **2** was reacted with an amino acid to give the phenylthioethoxycarbonyl (Pte) amino acid **3**, and then treated with methyl iodide and silver tetrafluoroborate to give the Pms amino acid **4**. Pms-Met-OH and Pms-Cys-OH can not be prepared by this method, however, because the sulfurs of Met and Cys are converted to the onium salt by treatment with methyl iodide.

To prepare Pms-amino acids including Pms-Met-OH and Pms-Cys-OH derivatives, we therefore designed the activated acylating onium reagent, 2-[phenyl-(methyl)sulfonio]ethyl-4-nitrophenylcarbonate tetrafluoroborate **6**, Pms-ONp. **1** was treated with methyl iodide and silver tertafluoroborate in acetonitrile to give 2-[phenyl(methyl)sufonio]ethanol tertafluoroborate **5** (yield 88%). 4-Nitrophenylchloroformate was reacted with **5** in acetonitrile to give **6**, Pms-ONp[4], in 71% yield. Pms-Met-OH **8**[4] was prepared using **6** in a mixture of 0.1% Triton X/water and acetonitrile (1/1) in the presence of pyridine with a yield of 61%. We also examined another synthetic route for Pms-Met-OH by the reaction of 2-[phenyl(methyl)sulfonio]ethoxycarbonyl chloride tetrafluoroborate(Pms-Cl) **7**, but the preparation of **7** by the reaction of **5** and phosgene failed because of the poor solubility of **5** in organic solvents. In addition, **7** could not be prepared in aqueous solvents such as aqueous acetonitrile and aqueous tetrahydrofuran because **7** and phosgene are unstable in aqueous media.

Keywords: 2-[phenyl(methyl)sulfonio]ethyl-4-nitrophenylcarbonate tetrafluoroborate; water-soluble *N*-protecting group; solid-phase synthesis; Met-enkephalin amide.

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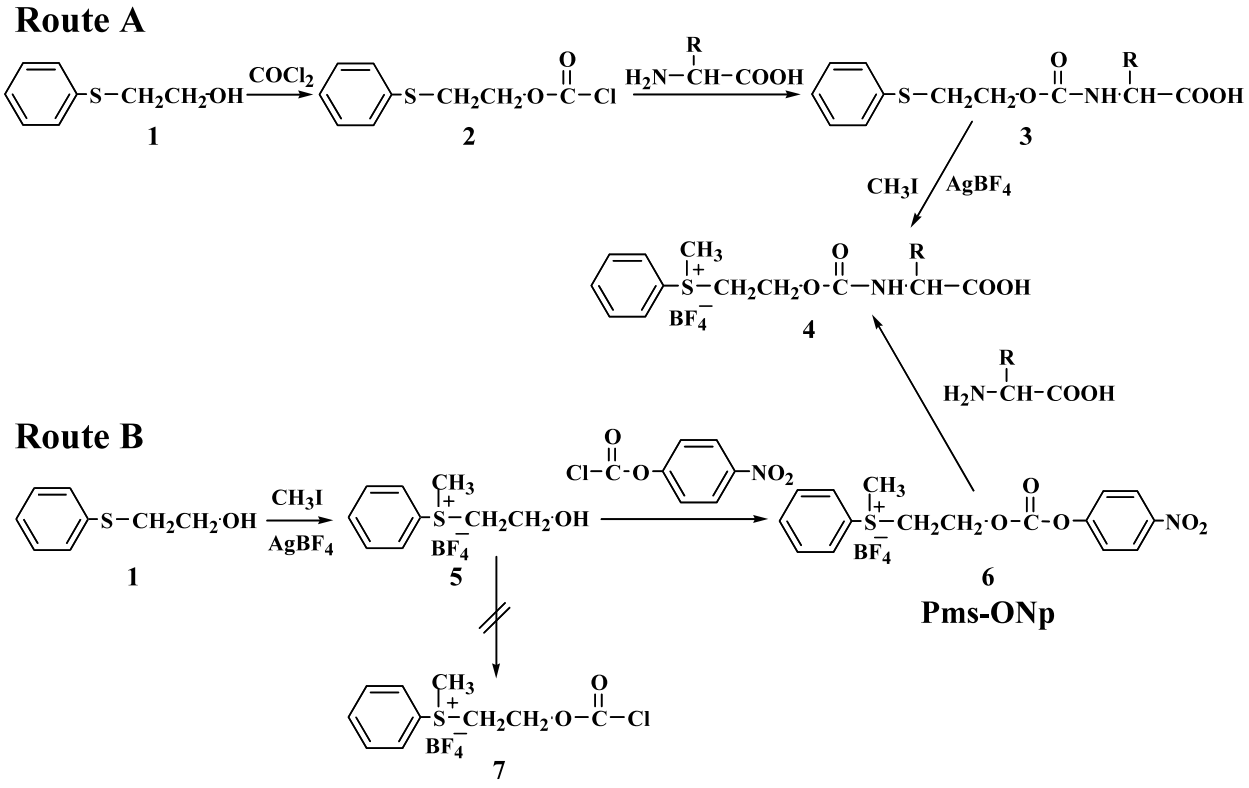

Scheme 1. Synthetic scheme for Pms-ONp and Pms-amino acids. Route A: Previous synthetic scheme for Pms-amino acids.[2] Route B: Present synthetic scheme.

N-Pms-*S*-acetoamidomethylcysteine [Pms-Cys(Acm)-OH] **9**[4] and *N*-Pms-*S*-tritylcysteine [Pms-Cys(Trt)-OH] **10**[4] were also prepared in the same manner. The yields of **9** and **10** were 25% and 68%, respectively. Whereas **8** and **9** were readily soluble, **10** was sparingly soluble in water. **10** was soluble in aqueous organic solvents, such as 50% acetonitrile and 50% dimethylforamide. Thus, the hydrophobicity of the trityl group is greater than the hydrophlicity of the Pms group in **10**.

Recently, a few resins that swell with water have become commercially available. To evaluate peptide synthesis using Pms amino acids and such resins, Met-enkephalin amide was prepared by the solid-phase method in water according to the protocol in Table 1. Previously,[2] we studied preparation of Leu-enkephalin amide on a poly(ethylene glycol)-grafted Rink amide resin[5] using Pms-amino acids in water. Here we selected a crosslinked ethoxylate acrylate resin (CLEAR, purchased from Peptides International) that has been reported to swell not only with organic solvent, but also with water.[6] The water-soluble carbodiimide (WSC), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride,[7] was used as a coupling reagent and *N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB)[8] was added as an additive to accelerate the coupling reaction. We used 0.5% Triton X in water as the solvent and removed the Pms group by treatment with 0.01 N aq. NaOH. Synthetic Pms-Tyr-Gly-Gly-Phe-Met-CLEAR resin was treated first with 0.01 N aq. NaOH, and then by a mixture of trifluoroacetic acid, thioanisole and ethanedithiol (94/3/3) to liberate the synthetic Met-enkephalin amide, H-Tyr-Gly-Gly-Phe-Met-NH_2. The HPLC profile of the crude peptide is shown in Figure 1A. The peptide was purified by preparative HPLC. The yield calculated from the used CLEAR resin was 29%.[4] Minor peaks were observed before and after the main peak of Met-enkephalin amide. The early peaks contained deletion peptides (such as Tyr-Gly-Phe-Met and Gly-Gly-Phe-Met), whereas the later peaks contained non-peptide compounds that might have derived from the used resin. For comparison, Met-Enkephalin amide was also prepared by the solid-phase method in water using the poly(ethylene glycol)-grafted Rink amide resin, and the HPLC profile of the crude product is shown in Figure 1B. Again, minor peaks were observed before and after the main peak of Met-enkephalin amide. The early peaks contained deletion peptides (such as Tyr-Gly-Phe-Met and Gly-Gly-Phe-Met) and the later peak at 24 min contained

Table 1. Synthetic protocol for the solid phase synthesis of Met-enkephalin amide in water

Steps	Reagents	Reaction time
1	0.2% Triton X/H_2O	3 min × 6
2	Pms-amino acid (4 equiv.), WSC (4 equiv.) HONB (4 equiv.) in 0.2% Triton X/H_2O	2 h × 2
3	0.2% Triton X/H_2O	3 min × 6
4	H_2O	3 min × 2
5	0.01N aq. NaOH	3 min × 2
6	H_2O	3 min × 3

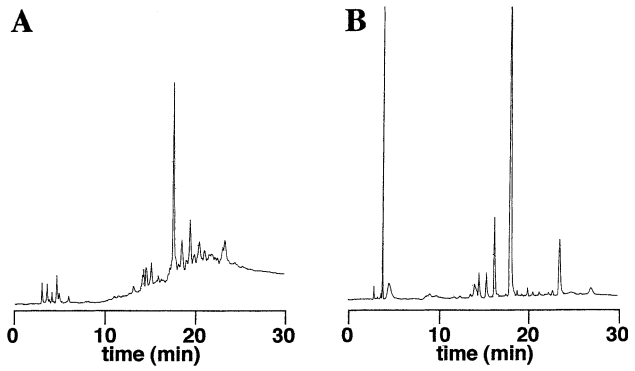

Figure 1. HPLC profiles of synthetic crude Met-enkephalin amide synthesized by the solid-phase method in water. A: Crude Met-enkephalin amide prepared on CLEAR resin. B: Crude Met-enkephalin amide prepared on the poly(ethylene glycol)-grafted Rink amide resin. The main peaks at 18 min in the eluates in both A and B contained Met-enkephalin amide. Column, DAISOPAK SP-120-5-ODS-B (4.6×250 mm). Flow rate, 1 ml/min. Eluent, CH_3CN/H_2O containing 0.05% TFA. Gradient: 10/90→50/50 (20 min). OD at 220 nm.

thioanisole. After purification by HPLC, the yield of Met-enkephalin amide prepared on the poly(ethylene glycol)-grafted Rink amide resin was 32%. The poly(ethylene glycol)-grafted Rink amide resin swells better than CLEAR resin in water, which might account for the slightly higher yield of Met-enkephalin amide prepared on the poly(ethylene glycol)-grafted Rink amide resin. The two synthetic Met-enkephalin amides (prepared on either type of resin) were verified by comparison with the HPLC of authentic Met-enkephalin amide prepared by the traditional fluorenylmethoxycarbonyl (Fmoc) group-based[9] solid-phase method using organic solvents. The yield of Met-enkephalin amide prepared by the Fmoc group-based solid-phase method was 76%.

The new reagent, Pms-ONp, was designed to prepare Pms-amino acids including sulfur-containing amino acids. Because the reagent is a crystalline compound and can be kept stable in a refrigerator, the preparation of Pms-amino acids is much easier with Pms-ONp than it is with the former method (route A, Scheme 1). Met-Enkephalin amide was successfully synthesized in water on both the CLEAR resin and the poly(ethylene glycol)-grafted Rink amide resin, although the yields were lower than that of Met-enkephalin amide prepared in organic solvents. We have examined the potential of the CLEAR resin and the poly(ethylene glycol)-grafted Rink amide resin for solid-phase synthesis in water, but neither resin swelled sufficiently in water. Future work should aim to develop a new resin that swells more than these two resins in water.

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4. **Pms-ONp 6**: mp. 134°C (decomp.), Tof-MS m/z: 334.2 (M^+, $C_{16}H_{16}NO_5S$ requires 334.4), ^1H NMR (400 MHz, CD_3CN): δ 8.30 (2H, d-like, $J=9.3$ Hz); 7.99 (2H, dd-like, $J=8.5$, 1.2 Hz); 7.85 (1H, tt-like, $J=7.4$, 1.2 Hz); 7.75 (2H, dd-like, $J=7.4$, 8.5 Hz); 7.43 (2H, d-like, $J=9.35$ Hz); 4.67 (1H, m); 4.45 (1H, m); 4.04 (1H, m); 3.96 (1H, m); 3.32 (3H, s). **Pms-Met-OH 8**: $[\alpha]_D^{24}$ −33.6° ($c=1.0$, CH_3CN), Tof-MS m/z: 344.3 (M^+, $C_{15}H_{22}NO_4S_2$ requires 344.5). **Pms-Cys(Acm)-OH 9**: $[\alpha]_D^{24}$ −20.1° ($c=1.0$, CH_3CN), Tof-MS m/z: 387.5 (M^+, $C_{16}H_{23}N_2O_5S_2$ requires 387.5). **Pms-Cys(Trt)-OH 10**: $[\alpha]_D^{24}$ +7.3° ($c=1.0$, CH_3CN), Tof-MS m/z: 558.5 (M^+, $C_{32}H_{32}NO_4S_2$ requires 558.7). **Met-enkephalin amide**: $[\alpha]_D^{24}$ −6.7° ($c=0.8$, 20% CH_3CN/H_2O), Tof-MS m/z: 573.7 [$(M+1)^+$, $C_{27}H_{36}N_6O_6S$ requires 572.7]. Amino acids analysis, Tyr 1.01; Gly 1.00; Phe 0.95; Met 0.93 (average recovery 94%).
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